concentrations approximating their physiologic concentration in cartilage. As well, there is a differential inhibition of organic phosphatase, pyrophosphate hydrolase, and CPPD crystal dissolution activity by these amino acids. In OA, there is a potential for bone ALP to be down regulated by increasing the concentration of inhibiting amino acids or their analogues. In CPPD, sequestration of inhibitory amino acids may permit sufficient ALP activity to facilitate CPPD crystal dissolution.

204 ESE-1 REGULATES BASAL EXPRESSION OF MMP-13 IN CHONDROCYTES

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Purpose: Among the matrix metalloproteinases (MMPs), MMP-13 plays a major role in osteoarthritis (OA) due to its-rate limiting role in the collagen degradation. MMP-13 expression is regulated by a variety of transcription factors, including members of the ETS family such as ETS-2 and Elk-1, but the precise transcriptional mechanism is not completely understood. ESE-1 is expressed in non-epithelial tissues after treatment with inflammatory cytokines and we reported recently that ESE-1 represses the type II collagen gene (COL2A1) in human chondrocytes. These observations suggest a fundamental role of ESE-1 in cartilage degeneration and suppression of repair. The aim of this study was to determine whether ESE-1 participates in the MMP-13 transcriptional regulation in chondrocytes.
Methods: The binding of in vitro-translated ESE-1 to the MMP-13 promoter was analyzed by electrophoretic mobility shift assay (EMSA) and supershift analysis. For knockdown of ESE-1 expression, the human chondrocytic C-28/I2 cells were transfected with a pool of four individual siRNA oligonucleotides against ESE-1 or control nonspecific siRNA. After incubation for 72 hours, the knockdown was confirmed by real time PCR and Western blotting, and MMP-13 mRNA was analyzed by real time PCR. MMP-13 protein in Ese1 (Elf3)-deficient mice was addressed by immunohistochemical staining.
Results: Three highly conserved ETS binding sites were revealed in the proximal region of the MMP-13 promoter. By EMSA and supershift analysis, we detected specific binding of ESE-1 to all three sites in the proximal MMP-13 promoter, with stronger binding to the site containing 2 tandem ETS sites. These results suggested that ESE-1 could be regulating the MMP-13 promoter activity. By using specific siRNA oligos, we knocked down the ESE-1 expression in C28/I2 cells. Real time PCR analysis revealed lower MMP-13 basal expression associated with ESE-1 knockdown. This result suggests that ESE-1 is necessary for maintaining the MMP-13 basal expression levels. Finally, the immunohistochemical analysis of the Elf3-deficient mouse cartilage revealed low or undetectable MMP-13 protein levels in comparison with age-matched wild type cartilage samples, although type II collagen staining was similar with no evidence of proteoglycan loss.
Conclusions: Our previous results highlighted ESE-1 as an important transcriptional regulator of cartilage degenerative processes. After induction, ESE-1 transactivates both NOS2 and COX2 promoters by interacting with NFκB. In addition, we showed recently that ESE-1 represses the COL2A1 in human chondrocytes and that ESE-1 immunostaining is high in human OA cartilage, whereas it is not present or undetectable in control cartilage. In this work we show that ESE-1 binds directly to the MMP-13 proximal promoter region and that knocking down the ESE-1 expression leads to decreased MMP-13 basal expression levels. These results suggest that, among others, ESE-1 is a key factor for maintaining the MMP-13 basal expression. Moreover, our finding that MMP-13 protein levels are decreased or absent in the cartilage of Elf3-deficient mice further reinforces the role of the ESE-1 in maintaining MMP-13 basal expression. However, the lack of evidence of collagen degradation in wild type samples indicates that activation of the proenzyme requires additional stimuli. Altogether, our results suggest a fundamental role for ESE-1 in controlling MMP-13 gene expression in chondrocytes. Further work will be required to determine its role in progressive cartilage loss during OA.

205 X-RAY MICROSCOPY OF JOINTS: SUBCHONDRAL BONE MINERAL STRUCTURE IN OSTEOARTHRITIS

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Purpose: Increased subchondral bone thickness, altered bone mineral density distribution, and trabecular microfractures are characteristic morphologic features of osteoarthritis. However on the microscopic scale, (μm), the bone mineralization patterns remain to be elucidated. To develop a better understanding of subchondral bone mineralization in osteoarthritis and to explore the utility of high voltage, high resolution x-ray microscopy in resolving bone structure, we studied the articular bone plate and subchondral bone trabeculae in ten cases of osteoarthritis using CPPD crystal deposition disease and osteoporosis cases as control tissues.
Methods: Five mm coronal blocks were taken from the femoral condyles at cross sections where osteoarthritis was maximal and where articular cartilage was still present. These blocks were adjacent to blocks taken for routine diagnostic analysis. The formalin-fixed blocks were studied using a Comet Feinfocus 160.25 x-ray microscopy system (focal spot <1 μm). For this study, magnification was set at the highest practical level to obtain clear images, projection magnification = 28×. X-ray exposures were optimized to characterize the bone mineral structure (80 kV, 45 uA). Magnification was calibrated using a copper electron microscope grid diameter = 3 mm, with grid line width = 35 μm.
Results: Samples clearly showed resolution of features less than 10 μm in width and demonstrated different mineralization density within bone structures. Figure 1 shows a sample range of the initial morphologic observations. The osteoporosis control, Fig. 1.3 shows subchondral bone with similar density to that of the bone trabeculae (bars) as well as extensive but focal, trabecular microfractures with increased mineralization (arrow). Samples from osteoarthritis patients, Fig. 1.2 and 1.3, demonstrated increased bone mineral density within the subchondral plate. However, Fig. 1.3 showed extensive trabecular microfractures with highly mineralized density compared to normal trabeculae whereas Fig. 1.2 did not. In Fig. 1.4 from the calcium pyrophosphate crystal deposition disease, the image shows a morphologic pattern more closely resembling osteoporosis Fig. 1.3 than osteoarthritis.
Conclusions: These studies show that X-ray microscopy is a promising tool to delineate subsets of subchondral bone response in osteoarthritis. The observations confirm that subchondral osteosclerosis in osteoarthritis is related to increased bone mineral density of the subchondral bone plate irrespective of bone thickness. However, trabecular microfractures with increased mineralization density occurs only in a portion of patients with osteoarthritis. This indicates that the subchondral bone trabecular structure and mineralization distribution in osteoarthritis is heterogeneous. Most likely, this reflects different types of osteoarthritis with differential bone mineralization response, rather than different stages of the disease.

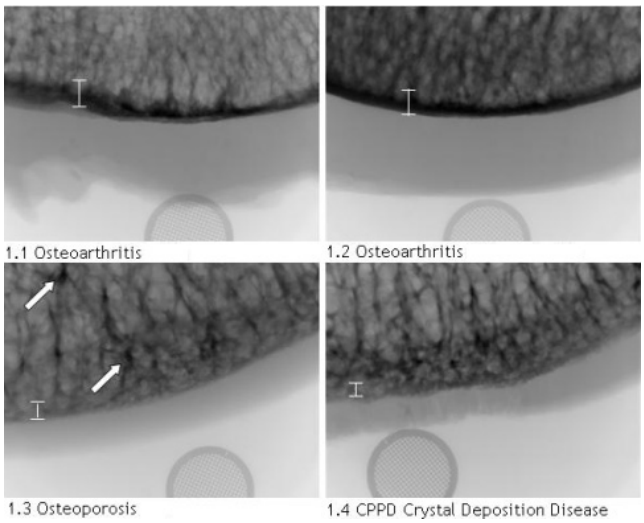

Figure 1.

206 CHONDROCYTE TELOMERE LENGTH DECREASES AS THE LEVEL OF CARTILAGE DEGENERATION IN HUMAN OSTEOATHRITIC KNEES INCREASES

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Purpose: Research in osteoarthritis (OA) has in recent years suggested a relationship between chondrocyte telomere length and severity of OA. We